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HORIZONTAL AND AMACRINE CELLS OF THE RETINA: PROPERTIES AND MECHANISMS OF THEIR CONTROL OVER THE BIPOLAR AND GANGLIONIC CELLS

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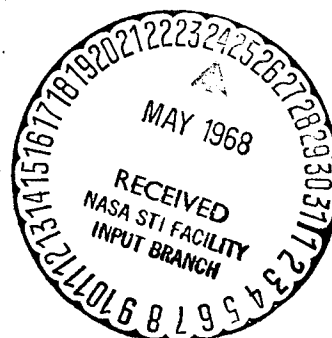
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HORIZONTAL AND AMACRINE CELLS OF THE RETINA: PROPERTIES AND MECHANISMS OF THEIR CONTROL OVER THE BIPOLAR AND GANGLIONIC CELLS

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ABSTRACT. A general review is presented of the morphological and electrophysiological findings which support our theory concerning the horizontal and amacrine cell control of the excitability and coding mechanisms of the retina.

Evidence is presented for 1) the existence of a functional contact ("transferapsie") between horizontal cells and amacrine cells, which is different from the electrical or chemical synapse; 2) the nonionic nature of the S-potentials, which are intimately dependent on plasma membrane respiration. Various proofs are given for the fact that horizontal cells exert their nonspecific excitability control on the bipolar cell dendrites through a transferaptic influence. By analogy, certain amacrine cells exert a specific control, wavelength- or space-dependent, on the dendrites of certain ganglion cells coding color, black-white or space.

... Hypotheses in science serve an important /254*
purpose, even if they are erroneous. This pur-
pose is not always to formulate a truth, but to
mark a path for investigation. They are above
all great awakeners of souls, for they stir the
moral atmosphere (that dead sea of routine,
fatal to all progress) ...

Ontologically the retina is a derivative of the forebrain. In its adult state it forms part of the gray matter, resembling in structure the cerebral cortex. The structural makeup of the retina in fishes is fundamentally like the retina of mammals and man, and in certain species there are giant-size horizontal and amacrine cells, whose function can thus be studied at the cellular level, a study which thus far has been technically impossible in other tissues.

The horizontal cells were classified by Cajal (1893, 1899-1904, 1952-1955) as "short-axon cells", and the amacrine cells were so named because they lack an axon. These cells have stimulated the imagination of more than one investigator. It was Cajal (1899-1904) who suggested the possible physiological role

*Numbers in margin indicate pagination in foreign text.

of the short-axon cells in cerebral function, pointing out that they could be the elements responsible for the higher functions ("memory, ideation, judgment", etc.) and that possibly they might exercise a control over the long-axon neurons, the function of the latter being to carry information in the form of pulses over long distances. Cajal (1899-1904) noted that the short-axon cells were very abundant in the gray matter of the higher cerebral centers, but that "such elements are almost entirely lacking in the spinal cord and the medulla oblongata" (Vol. II, p. 447), which are centers that carry out simpler reflex functions. The horizontal cells of the retina, according to Cajal's view, are "a repository of nerve energy designed to strengthen the impression and give it the potential necessary for its propagation to the centers" (Vol. II, p. 644). In general the short-axon cells are "condensers or accumulators of nerve energy" (Vol. II, p. 859). "The arrival of a current along a centripetal fiber will initiate the discharge of the subordinate elements of the short-axon cells, and this will contribute to an increase in voltage of the pulses traveling along the chain of long-axon neurons" (Vol. II, p. 446).

Up to the present time there has been no concrete information concerning the function of the short-axon and amacrine cells, except for the studies on the electrical responses recorded in the horizontal cells and certain amacrine cells of the retina which were initially carried out in this laboratory (Svaetichin, 1953, 1956). Such responses were designated "S" potentials by K. Motokawa (see Tomita et al., 1959). As a consequence of the experiments carried out in this laboratory (see below), it is possible to reconcile our data and Cajal's ideas concerning the function of this class of cells in the retina.

Strictly on the basis of morphology it is difficult to classify the horizontal and amacrine cells as either neurons or glial cells (Svaetichin et al., 1965 a, b; Selvin de Testa, 1966; Parthe, 1967 a, b; O'Daly, 1967 a, b). The absence of Nissl substance, the lack of the typical axon (Parthe, 1967 a, b), the elevated content of glycogen (Parthe, 1967 a, b; O'Daly, 1967 b), and the presence of the enzyme carbonic anhydrase (Parthe, 1967 b) are the traits which identify these cells with the glial group rather than with the neurons. Because of the lack of unequivocal morphological data permitting their precise classification, the horizontal and amacrine cells have been distinguished physiologically as "control cells" (Svaetichin et al., 1965 a, b) on the basis of their functional characteristics, which are totally different from those presented by the long-axon neurons.

In previous publications of this laboratory the possibility was suggested (Laufer et al., 1961; Mitarai et al., 1961; Svaetichin et al., 1961; Vallecalle and Svaetichin, 1961, Svaetichin et al., 1965 a, b; and Negishi and Svaetichin, 1966 a, b, c, d) that the retinal cells generating S potentials exercise a control over the bipolar and ganglionic cells of the retina, and that, analogously, the cerebral function in general might be subject to similar mechanisms of control. The existence of control systems in the retina was discussed extensively in similar terms by Lipetz (1963). The postulated theory has been confirmed on all points by studies concluded subsequently. In the limited space of this paper we shall present and discuss some of our experimental results.

The classical studies of Cajal on the structure of the nervous system in general and the retina in particular (Cajal, 1893) are fundamental and almost

incapable of improvement. Without complete charts of the microscopic struc-
ture, understanding of the function would be impossible. The three-dimensional
diagram in Fig. 1 represents the structure of the retina of a telecost (Mugel bra-
siliensis), and was drawn by C. Muriel on the basis of the results of microscopic
studies by Parthe (1967 a, b) using the methods of Golgi, Bielschowsky, etc.
Thanks to the studies of O'Daly (1967 a, b), who used electron-microscope and
electron-histochemical methods, new detailed information is appearing on the
ultrastructure of the different elements of the retina as well as concrete infor-
mation concerning a new type of contact between cells. The electron-microscope
study of the teleost retina by G. Villegas (1960) demonstrated that the horizontal
cells establish membrane-to-membrane contact by means of the lateral segments
of their expansions establishing a structural unit. Yamada and Ishikawa (1965)
described contacts, with fusion of membranes, between the lateral processes of   /260
the horizontal cells of the retina of fishes. O'Daly (1967 a, b) has identified this
structure, not only between horizontal cells, but also between amacrine cells,
and between these cells and the bipolar and ganglionic cells. In this contiguous
area of contact between the cells situated in a single layer generating S poten-
tials, the two plasma membranes in lateral contact are united, always excepting
the synaptic vesicles in the vicinity of these contacts.

The S Potentials Depend on Nonionic Mechanisms. Transferapse.

The existence of nonsynaptic functional contacts between cells generating
the S potential, and between S cells and neurons, was postulated basically on
electrophysiological studies (Laufer et al., 1961; Mitarai et al., 1961; Svaetichin
et al., 1961; Svaetichin et al., 1965 a, b; Negishi and Svaetichin, 1966 a, b, c,
d). This type of functional contact was designated "transferapse": "Intercellular
transfer of excitation ... occurs only at specialized regions ('transferapse'), where
the apposed plasma membranes are partly fused"; Svaetichin et al., 1965 a, p. 260).
Recently O'Daly (1967 a) has demonstrated a high concentration of ATPase, which
does not correspond to the cation transport type, at the transferaptic contact and
a small concentration all along the plasma membrane. A similar ATPase was
observed by Novikoff et al. (1966) in the areas of contact between long-axon
neurons and their neuroglia satellite cells. The transferapse laterally joins the
horizontal and amacrine cells, in this way forming functional strata which ex-
tend throughout the entire retina (Figs. 1 and 2). By means of the transferaptic
contact the horizontal and amacrine cells can influence the excitability of the
long-axon neurons.

The majority of the ATP produced by oxidative degradation of glucose in
the cells is formed inside the mitochondria, but the mitochondrial membrane
is relatively impermeable to ATP (Lehninger, 1965). Because of its specificity
it is probable that ATPase is localized only at places where ATP exists. Hence
the existence of ATPase close to the membrane of the S cells suggests that ATP
forms there also. The presence of O_2 is necessary for the generation of the S
potentials, which are blocked by CO, HCN, N_3H (Negishi and Svaetichin, 1966 a),
and malonate, but not by DL-glyceraldehyde (Santamaria et al., 1967). Changes
of temperature markedly influence the behavior of the membrane potential of
the S cells, which reflects an intimate relation between enzyme systems rather
than between ionic diffusion equilibria (Laufer et al., 1961; Fatehchaud et al.,
1965; Svaetichin et al., 1961, 1965 a, b; Negishi and Svaetichin, 1965, a, b, c,
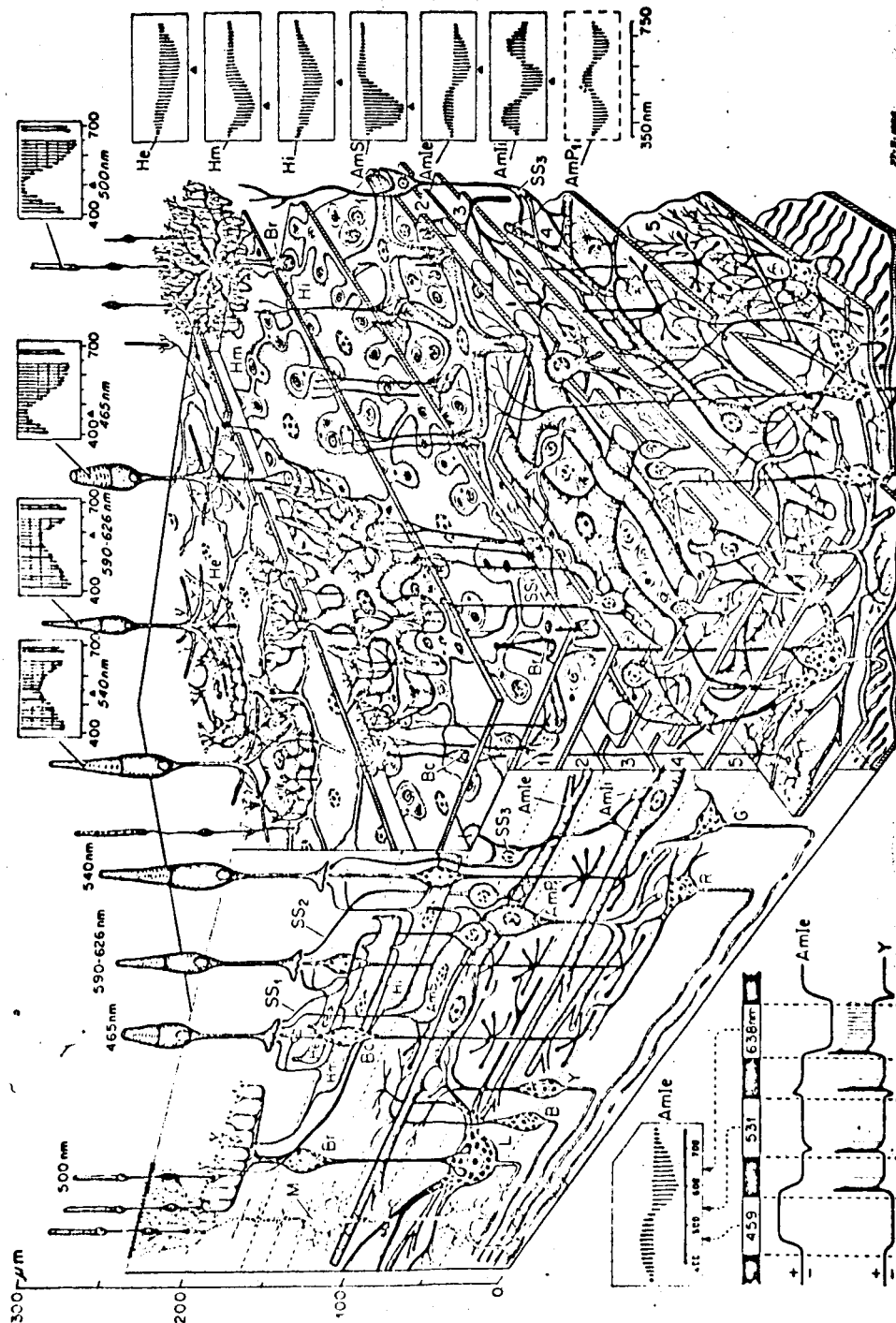
Figure 1. Semischematic Drawing of the Structural Organization of
a Teleost Retina (Mugil Brasiliensis), Drawn by C. Muriel on the Ba-
sis of Microscopical Studies by Parthe (1967 a, b), Combined with
Electrophysiological and Photopigment Absorption Data.

The origins of the different S-potentials are indicated. Three layers
of horizontal cells below the external plexiform layer: He: external;

Hm: medial and Hi: internal horizontal cells. The lateral processes of the horizontal cells in a given layer are in contact with each other and form a continuous network. The inner plexiform layer is divided in 5 strata indicated 1-5. Below the horizontal cells a continuous network of stellate amacrine cells: AmS. Cells of an enormous size, which form networks in strata 2 and 4, correspond to Cajal's (1893) interstitial amacrine cells: Amle, Amli. The cell bodies of the piriform amacrine cells: AmP are located among the AmS and Amle. The large synaptic endings of the rod-bipolar cells (Br) are found directly on the cell body of the L-type of ganglion cell, the dendrites of which are distributed in strata 1-5. The cone-bipolars of most vertebrates have collaterals, which are found within certain strata of the inner plexiform layer, as though they would be distributed according to a given plan. Collaterals have not been observed in the teleost retina, but the synaptic terminals of the cone-bipolars are distributed in a similar way in certain strata. The cone-bipolars have been provided with collaterals in order to simplify the schemes in Figs. 1 and 2, in our attempts to offer an interpretation of the retinal processing system which produces the opponent color and black-white signals. The microspectrophotometrical recordings of the photopigment absorption (Svaetichin et al., 1965 b) are placed close to the corresponding three classes of cones and the rod; absorption maxima indicated by black triangles. To the right of the scheme are shown different types of "spectral response curves" which represent S potentials evoked by constant energy light stimuli of different wavelengths given at bottom: He, Hm, Hi from horizontal cells and AmS, Amle, Amli, AmP$_1$ from amacrine cells. Hyperpolarizing, cell inside negative responses downwards. In the left corner bottom, is shown spectral response curve Amle together with schematically drawn fast sweep recordings of responses from same cell evoked by the three given wavelengths (cf. record XIII, Fig. 2). The impulses which are superimposed on changes of the resting level in schematic drawing Y, correspond to intracellular recordings similar to record X, Fig. 2, which originate in a Y-type ganglion cell.

d). The membrane potential of the horizontal and amacrine cells can be displaced several hundred millivolts in either direction by electrical polarization (up to 350 mV, demonstrated by Negishi, 1967), without affecting the S-potentials induced by light (MacNichol and Svaetichin, 1958; Watanabe et al., 1959; Gouras, 1960; Murakami and Kaneko: see Tomita, 1965; Negishi, 1967). The change in membrane potential produced by electrical polarization does not affect the level of the membrane potential of nearby S cells (Murakami and Kaneko: see Tomita, 1965; Negishi, 1967), but a membrane response induced by a focus of light causes changes in the membrane potential of networks of S cells which cover a wide area /261 around this light focus projected on the retina. This fact shows clearly that the propagation of the S-potential in a particular network of S cells is an active process, which cannot be explained in terms of electrotonic propagation or cable theory. The manifest finding that the ionic flux of electrical current does not affect the behavior of the membrane of the horizontal and amacrine cells,

Figure 2. Structural Framework Based on the Information Pre-
sented in Fig. 1, Correlated with Photopigment and Electrophys-
iological Data, Representing a Synthesis of Our Attempts to
Analyze the Functional Organization of the Teleost Retina in
Terms of a Young-Hering Theory for Vision.

The cells are marked according to Fig. 1. The functional con-
nections between cells are indicated by symbols shown at top
of the scheme. a: Synapsis. b-d: Different kinds of transfer-

apses; b: S potentials propagation in two directions within a given horizontal or amacrine cell layer; c: opponent interaction between a pair of different amacrine cells (e.g., AmS-Amle); d: transferaptic contact between a dendrite and a horizontal o amacrine cell. To the right are shown electrical responses of different retinal structures to light stimuli. Record I: trans-retinal potential. II: Same as record I after action of NH_3, showing only the focal response of the receptors. III: Hypothetical bipolar responses which correspond to the b- and d-waves of the ERG. IV and V: Intracelluiar simultaneous recordings from Amle and Hi. VI and VII: Intracelluiar simultaneous recordings from dendrite of L-ganglion cell and Hi. VIII and X: Intracellular recording from type Y ganglion cell. XI: Intracellular recording from type B-W ganglion cell. XII: Extracellular recording from L type ganglion cell. Records I, II and XI were evoked by focal (at site of electrode) and nonfocal white light stimuli. The focal and nonfocal light spots were of diam. 0.25 mm and 1.5 mm apart in records I and II; in record XI spot diam. 1 mm and distance between spots 2 mm. Records III to X and record XII evoked by focal stimuli of wavelengths 459 and 638 nm. Numbers to the right of the recordings indicate the resting membrane potential. Calibration bar: 1 mV for records I and II; 20 mV for records IV-XI. Fast sweep records XIII obtained from Amle using the wavelengths indicated. Letters B, Y, B-W, G, R to the left indicate the strata of the inner plexiform layer in which the signals for blue, yellow, black-white, green and red are suggested to be processed.

in addition to the other facts mentioned above, demonstrates the presence of cellular elements in the retina which are functionally different from the long-axon neurons, although the mechanism of conduction of impulses in them requires a membrane excitable by electric (ionic) current. Therefore, in order to explain the propagation of excitation along the membrane of the S cells and across the transferapse in a particular network of S cells, it was necessary to postulate a nonionic molecular mechanism in the plasma membrane, which is intimately related to the transfer of electrons in the respiratory chains (Svaetichin et al., 1963; Svaetichin et al., 1965 a, b; Negishi and Svaetichin, 1966 a, b, c).

The excitatory or inhibitory post-synaptic potentials (EPSP or IPSP) are transitory processes of short duration (Eccles, 1964), while the change in the d.c. level of the membrane potential in the horizontal or amacrine cells stays at a constant amplitude during the light stimulation (Fig. 4, L and M). The membrane potential of these cells never shows the so-called "synaptic noise" or spontaneous synaptic potentials "in miniature"; the S-potentials, like the potentials of receptors, never appear spontaneously. On the other hand, the S-potentials do not undergo changes in connection with electrical polarization of the membrane (see previous paragraphs), which eliminates an ionic equilibrium potential, the membrane behaving like a linear ohmic resistance (Watanabe et al., 1959). The injection of Cl^- ions influences the IPSP's (Eccles, 1964) but affects neither the S-potentials of the depolarizing type nor those of the hyperpolarizing type. Evidently the S-potentials cannot be considered as post-synaptic

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potentials; and therefore the functional contacts which transfer the S potential from one cell to another in the same layer, as well as the functional contacts between the cells generating the S-potentials, with the receptors on the one hand and the bipolar and ganglionic cells on the other, should not be considered as synapses. The previous conclusions are in harmony with the ultrastructure studies of O'Daly (1967 a, b), and hence there is no doubt about the existence of a new type of functional contact in nerve tissue: the transferapse.

POST-SYNAPTIC POTENTIALS IN THE GANGLION CELLS

The intracellular recordings marked by the letter "d" in Fig. 3 were obtained in the outside zone of the internal plexiform layer. The potentials are transitory (like "saw teeth") and occur at the "on" (start) and "off" (stop) of the light stimulus, being accompanied sometimes by discharges of pulses (Fig. 3 A, B). These potentials have been observed by Laufer et al. (1961), by Villegas (1961), and by Lipetz (1963). The patterns of these responses do not depend on the wavelength of the light, nor does the distribution of the illuminated areas have a specific opposing influence on the membrane potential (cf. Fig. 2, Record IX). Numerous recordings have been made of this type of response under different experimental conditions, but only depolarizing (exciting) potentials have been observed. The spectral sensitivity covers the entire spectrum with a maximum in the middle of the visible range. These records possibly originate in the dendrites of the ganglion cells of type L (Fig. 1 and 2), which probably transmit information on brightness, which all classes of receptors collect. The so-called dendritic potentials ("d" in Fig. 3) show characteristics similar to the post-synaptic exciting potentials (EPSP) recorded for various long-axon neurons (Eccles, 1964). The "noise" on the records is probably due to spontaneous synaptic bombardment. Spontaneous transitory potentials of 10 to 20 mV. as well as spontaneous discharges of pulses occur when the retina is cooled 2-3° C below the ambient temperature.

MECHANISMS OF CONTROL IN THE RETINA

The retina is able to process the information received by the three classes of cones and the rods, coding signals of brightness, color, black-and-white, contrasts of space and time, etc., as well as to adapt its sensitivity continuously to an enormous range of illumination (10^{10}). It is highly improbable that these retinal functions are carried out without the presence of control mechanisms. The existence of a system of control in the retina was revealed by Vallecalle and Svaetichin (1961).

The retina is made up of chains of cells oriented radially: receptor-bipolar-ganglionic, which pass through the cell networks arranged tangentially: amacrine and horizontal (Fig. 1 and 2). The expansions of the horizontal and stellate amacrine cells are intermixed with the dendrites of the bipolar cells in the external plexiform layer, at the point where the bipolars receive information from the receptor. The internal plexiform layer is organized into 5 different strata (Fig. 1 and 2, tagged 1-5); in each stratum the expansions of particular amacrine cells are connected to the dendrites of certain types of ganglionic cells, at the point where these cells receive information from the corresponding bipolar cell. This structural organization suggests that the horizontal and amacrine cells act in some way on the signals which flow through the receptor-bipolar-
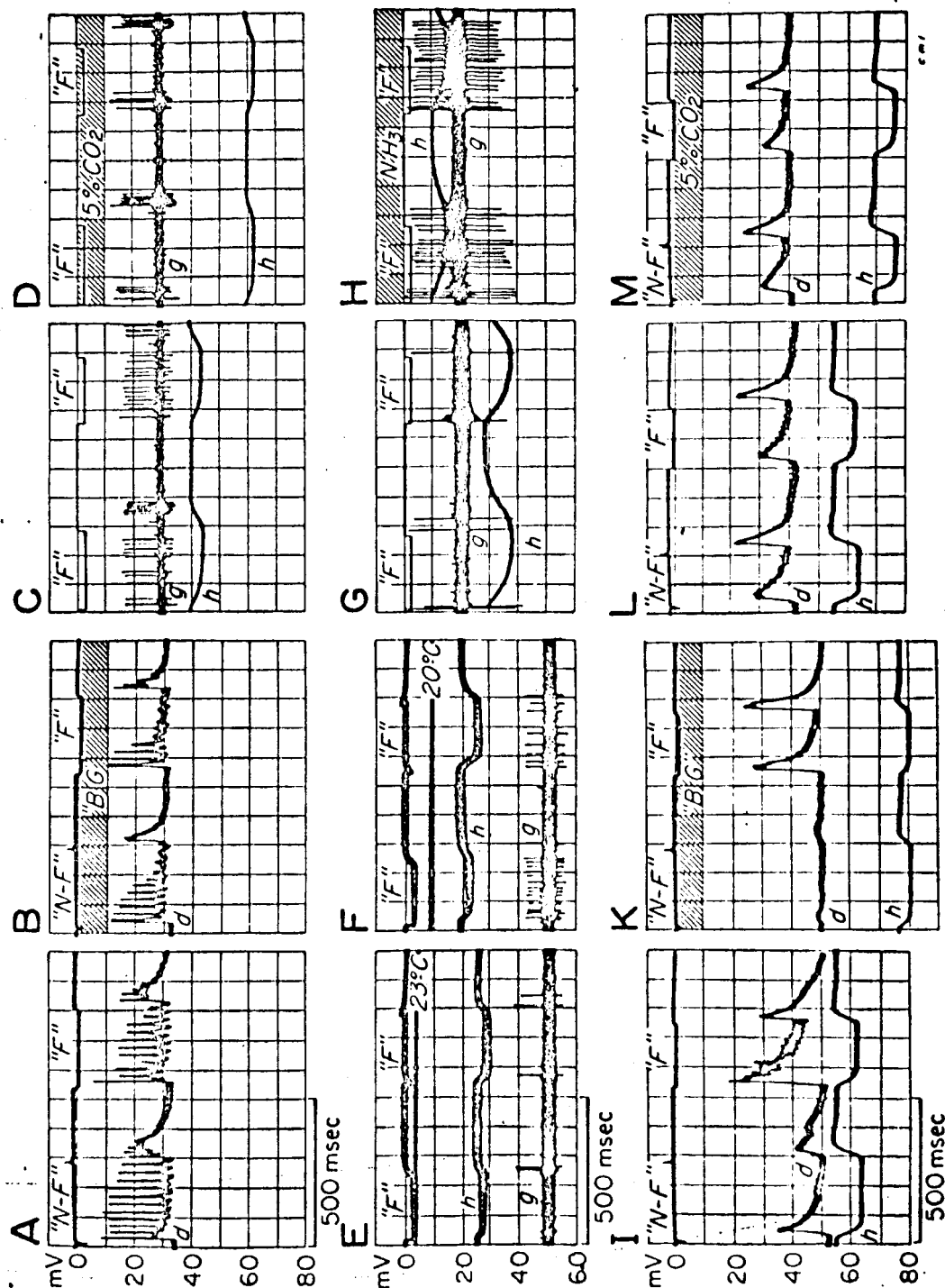
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Figure 3. Correlation Between Behavior of L-Type Ganglion Cell and
Horizontal Cell under Different Experimental Conditions.

AB and IK: Effect of background illumination; CD and LM: effect of 5%
CO_2; EF: effect of temperature changes; GH: effect of NH_3. Two beams
of light were focused from the vitreous side onto isolated retina; spot

diam. 1 mm and distance between spots 2 mm. The stray light was reduced by 80-90% by the use of two crossed polaroid filters mounted on diaphragms located at a distance of 0.5 mm below the retina; the light beams were polarized 90 degrees in respect to each other. The light spot at the site of the recording electrode is called "focal" ("F") and the second spot "nonfocal" ("NF"). The light intensities of the two spots were equal and showed an intensity 1000 times higher than the background light ("BG") used for illumination of the total retinal area. The moist chamber was circulated by O_2 or O_2 + 5% CO_2. d: Intracellular recording from dendrite of L type of ganglion cell. h: Intracellular recording from horizontal cell. Temperature recording in EF by means of microtermocouple in contact with the retina.

ganglionic cell chain, and will finally move as developed information to the corresponding brain center. The electrophysiological studies of the S-potentials fully confirm the theory that the horizontal and amacrine cells control the information that is propagated through the bipolar-ganglion cell chain. In our view it is thus impossible to find any other rational explanation for the morphological and functional facts mentioned.

Under certain experimental conditions (change of temperature, percent CO_2, etc.) sinusoidal oscillations are observed in the membrane potentials of the S cells at a frequency that is almost constant. The dependent relationship which exists between the frequency and the amplitude shows that the system contains nonlinear elements (Fig. 4 N). These oscillations can be spontaneous, produced or suppressed by light stimulation. Such oscillations have never been observed in the receptor potentials, a fact which suggests that the oscillations are created by interactions between cells which process information received from the receptors. These oscillations are probably due to a "ringing" of the retinal control systems, as was suggested by Vallecalle and Svaetichin (1961). The presence of oscillations does not prove the existence of control, but on the other hand the oscillations are always inherent in a control system. In a physical control system negative feedback causes the output signal to be less sensitive to the fluctuations responsible. An increase of background illumination ("BG") or of percent CO_2, which causes a reduction in the excitability of the retina (see below), also reduces the "noise" level, as can be observed in the recordings from ganglion cells, which are the output of the retinal system (Fig. 3, AB, CD, IK, and LM).

The control system of the retina (Vallecalle and Svaetichin, 1961) is composed of the following elements: 1) The input receptor, which gives a graduated square-wave response related linearly to the intensity of the light; 2) the forward conduction line, consisting of the bipolar-ganglionic cells, which is radially oriented and conducts the signal on an "all-or-none" basis without reduction, and 3) the horizontal and amacrine cells, which form tangential networks and produce graduated square-wave responses, which are propagated in decremental fashion; these tangential elements probably also constitute the power source of the control system. It is difficult, nevertheless, to present in physical terms the design of the control system in the retina.
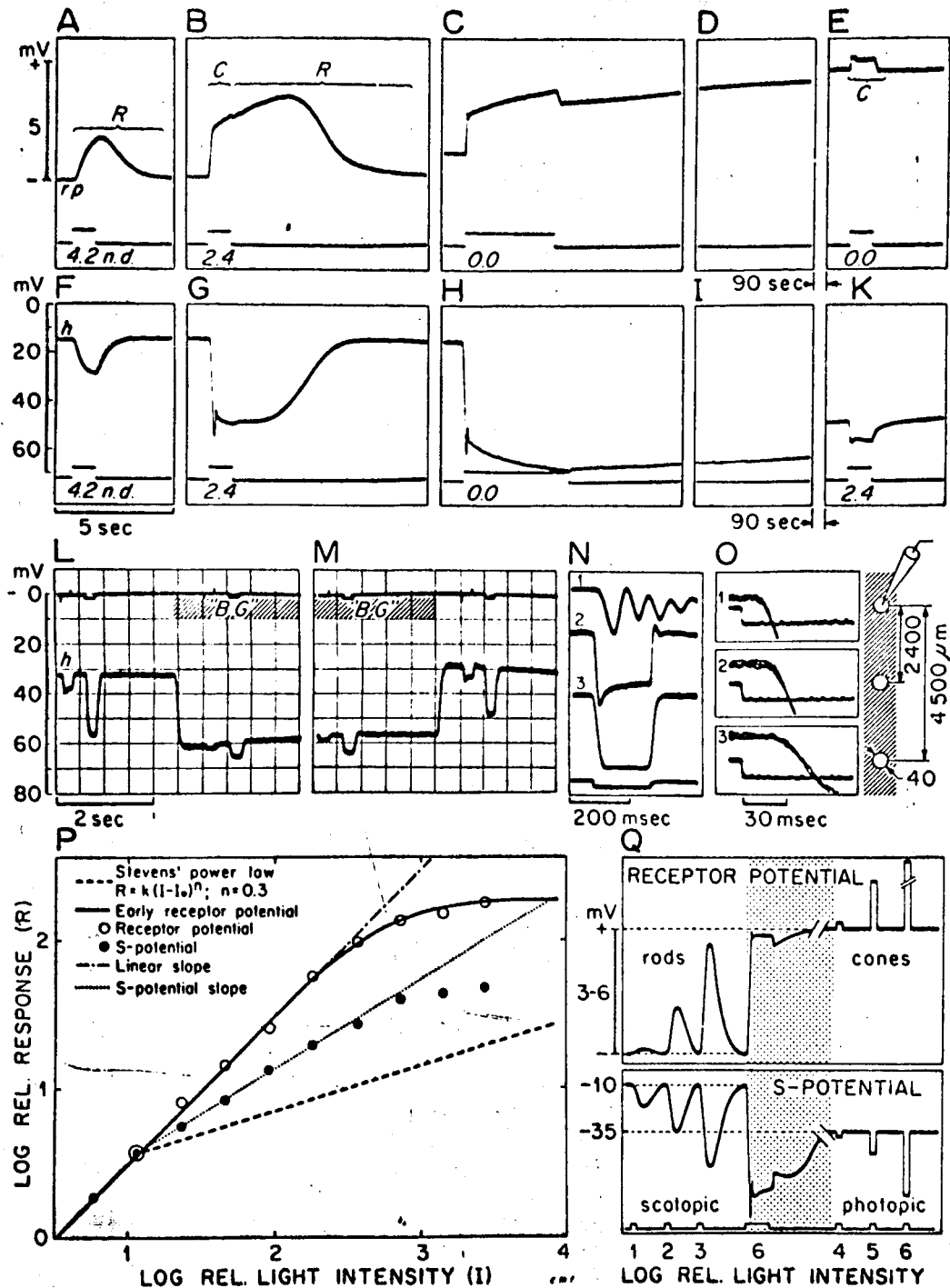
Figure 4. Comparison of Relations Between Response Amplitude (R) and Light Stimulus Intensity (I) of the "Receptor Potential" (Fatehchaud) et al., 1962), the "Early Receptor Potential" (Cone, 1965), the S-Potential and Stevens' (1961) Power Law, which States that the Magnitude of the Psychological Response (Brightness) Is a Power Function of the Magnitude of the Physical Stimulus (Light Intensity).

The receptor potentials are linearly related to the light intensity and show a slope of 1. At high intensities the deviation from linearity is due to saturation. The slope of the S-potential varies between 0.3 and

1.0 under different experimental conditions. A-E: Transretinal recording of receptor potential (rp) isolated by action of NH₃. A: activity of rods (R) in dark adapted retina and (E) activity of cones (C) after light adaptation; BD: shows transition from dark to light adaptation. The process of light adaption was induced by light stimuli of wavelengths 505 nm indicated at bottom trace; their relative intensities (n.d.) given in log units. B: Shows both rod (R) and cone (C) components of the receptor potential. Notice the transretinal DC-level change of nearly 5 mV in connection with the process of light adaptation. F-K: Intracellular recordings from one and same medial horizontal cell, which reflect the events which occur in the receptor layer during the process of adaptation to light. F: Scotopic response induced by rod activity; K: photopic response induced by cones. GI: Transition from scotopic to photopic state. Stimuli and their effect on adaptation, as in A-E. Notice the increase of the horizontal cell membrane potential from about 15 mV (F) to almost 50 mV (K). Q: Summarizes schematically the behavior of the receptor potential and the S-potential of medial and internal horizontal cells in the course of adaptation. LM: Record from a medial horizontal cell obtained by exposing the retina to focal and nonfocal light stimuli, to which background illumination ("BG") 1000 times less intense was added. N: Horizontal cells responses which show presence or absence of oscillations. O: Records obtained from a medial horizontal cell layer, evoked by a spot of light at different distances from recording electrode, in order to measure the velocity of spread of the S-potential (unpublished records from Mitarai et al., 1961).

NONSPECIFIC CONTROL

Weber's Law of Adaptation and Linearity of the Receptors

The "absolute threshold" of the retina, which is measured in the total absence of (zero) background illumination, is related to the concentration of the photopigment of the receptors; but the "incremental threshold", which is measured as a function of the level of background illumination, is not related to the concentration of photopigment (see Rushton, 1965). Weber's Law ($\Delta I/I$ = constant) states in simple form the well-known fact that the sensitivity of the eye decreases when prevalent level of background illumination increases and vice versa. Since Weber's Law is valid in general for all the sense organs, the basic mechanisms for the control of adaptation should be similar for all of them. This is the reason why Hecht's photochemical theory for adaptation sounds a discordant note.

Work in this laboratory (Fatehchaud et al., 1962), confirmed by Cone (1965), shows that the receptor potential retains a linear relation to the intensity over a considerable range of brightness (Fig. 4 P). Studies, using indirect methods, on the chromatic S-potentials give the same results with respect to the linearity of the receptors (Laufer, 1967). Hence Weber's Law of adaption cannot be explained on the basis of changes in the sensitivity of the receptor and it is necessary to invoke other mechanisms.

Each Layer of Horizontal Cells Constitutes an Adaptation Control Unit /268

The results obtained in this laboratory by Mitarai et al. (1961) would suggest that the horizontal cells exercise a control over the excitability of the

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bipolar cells, and that the change in sensitivity produced by the background illumination as well as Weber's Law can be explained in terms of such control.

Each of the three layers of horizontal cells operates as a functional unit independent of the others. Each layer shows a characteristic spectral response, the maximum of which corresponds to the maximum of the absorption curve of the photopigment of one of the three types of cones (Fig. 1, see Svaetichin et al., 1965 a, b). For this reason each layer of horizontal cells is related functionally to the type of cone which is exclusive to it. The functional independence of each of the three layers of horizontal cells and the functional interdependence of the cells of a single layer represent facts which agree with the anatomical observations showing the existence of transferaptic contacts in the horizontal-cell unions of a single layer (Yamada and Ishikawa, 1965; O'Daly, 1967 a, b).

In a particular layer of horizontal cells the response generated by a focal stimulus is propagated to nearby unilluminated areas at a speed of 0.35 m/sec (Mitarai et al., 1961). This propagation velocity, which was calculated by means of records obtained in different areas removed from the focal light point (Fig. 4 O), cannot be explained by synaptic contacts between one horizontal cell and another, since the successive synaptic delays would take much more time for propagation calculated on the basis of such recordings. Therefore the propagation of the activity in a layer of horizontal cells depends necessarily on the presence of transferaptic contacts.

The recording in Fig. 4 LM shows that a weak background illumination ("BG") produces an increase in the membrane potential of the horizontal cell and a marked reduction in the responses elicited by test light stimuli projected on a small area (1 mm in diameter) where the recording electrode is located, and the intensity of which is 1000 times greater than the background illumination. The effect produced by the background illumination is to increase the light intensity in the small test area by a factor of 0.001, without this insignificant increase influencing the sensitivity of the receptors. This experiment shows that marked changes in the responses of the horizontal cell to the test stimulus are produced by varying the background illumination, maintaining a constant receptor sensitivity. Obviously this effect, which does not depend on the characteristics of the receptor or the bipolar cell (see below), is due to spatial summation in a definite network of horizontal cells operating as a functional unit (Svaetichin et al., 1960). This behavior of the horizontal cell in connection with weak illumination of an extensive area of the retina is of fundamental importance for the interpretation of the results described farther on.

Correlation Between the Behavior of the Type L Ganglion Cell and the S-Potentials of the Horizontal Cells

In order to study the correlation between the S-potentials and the activity of the ganglion cells it was necessary to record simultaneously with two intracellular electrodes in both types of cells under different experimental conditions. The recordings shown in Fig. 3 are illustrative examples of these series of experiments. The S-potential of brightness (type L), the configuration of which resembles a square wave, cannot have any causal relationship to the process which generates the transitory dendritic potentials at the start ("on") and stop

("off") of the stimulus nor to the pulses superimposed on these potentials, which are recorded in the internal plexiform layer and which possibly originate in type L ganglion cells. This can be stated categorically, provided there are no anatomical contacts between horizontal and ganglionic cells.

The background illumination (white light) on a broad retinal area ("BG") at an intensity 3 logarithmic units less than that of the focal or nonfocal (Fig. 3 "F" and "NF") test stimuli influences the excitability of the ganglionic cell (Fig. 3 AB and IK), without the occurrence of detectable changes in the resting potential of the ganglion cell. An increase in the percent CO_2 produces a similar change in the excitability of the ganglionic cell (Fig. 3, CD and LM). The changes in excitability of the ganglion cell are reflected in the magnitude of the transient potentials (d) and in the patterns of pulse discharges, and they cannot be dependent either on the receptors, since these are linear (see above), or on the resting potential of the ganglionic cell, which does not vary. The horizontal and bipolar cells are the only structures remaining which can be considered as responsible for such changes in excitability. We come to this conclusion when we consider that the chromatic S-potentials (type C) do not undergo change in their resting level when the retina is illuminated with white background light (weak), excluding in this fashion the possible effects of the amacrine cells on the excitability of the type L ganglion cells. The changes in excitability of the type L ganglion cell induced by weak background illumination depend on a mechanism capable of adding and integrating information coming from a wide area, an effect which cannot be explained by means of the bipolars, since the latter are elements oriented radially and which do not form tangential networks. Although there can be no direct influence by the horizontal cells on the ganglion cells, there is a clear correlation between the behavior of membrane potential of the horizontal cell (see Fig. 3 and 4 LM) and the changes in excitability observed in the recordings from the type L ganglion cells when illuminated by weak and diffuse light. Hence the networks of horizontal cells should have some sort of influence on the excitability of the bipolar cells, which connect the receptors with the ganglion cells. The hyperpolarization of the membrane of the horizontal cell is correlated with a decrease in excitability, as observed in the behavior of the type L ganglion cell (Fig. 3, CD, IK, and LM), while a depolarization of the membrane of the horizontal cell is accompanied by an increase in the excitability of the L ganglion cell (Fig. 3, EF and GH). The hyperpolarization of the membrane of the horizontal cells which causes the increase in excitability seems to be related to a transferaptic depolarization of the dendrite of the bipolars. /270 We come to this conclusion because the nonfocal stimulation produces a transretinal square wave, negative on the receptor side (Motokawa et al., 1959; Fig. 2, Recording I, second response). This wave is not generated by the receptors, because their activity is strictly focal (Mitarai et al., 1961), but by an electrical dipole oriented radially, the potential of which varies at one pole in relation to the other. Such orientation and behavior fit the bipolar cells. The depolarization in the form of a square wave which occurs in the dendrites of the bipolars and which persists while the light stimulus lasts, cannot be considered as a postsynaptic potential but as a transferaptic influence induced by the horizontal cells. Further evidence for this is that the transretinal square wave of nonfocal origin disappear (Fig. 2, Recording II, second response absent) simultaneously with the elimination of the responses of the horizontal cells to the light stimulus by exposing the retina to minimal quantities of NH₃ vapor (Negishi and Svaetichin, 1967 a).

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It should be noted that suppression of the activity of the horizontal cell unmasks the potential of the receptors (Mitarai et al., 1961; Fatehchand et al., 1962) elicited by the focal stimulus (Fig. 2, Recording II; the first response is now greater).

An unusual circumstance is that carbonic anhydrase is lacking in the bipolar and ganglion cells and is localized in the horizontal cells and certain amacrines (Parthe, 1967 b). The hydration of CO_2 in the tissues occurs at a speed approximately a thousand times greater in the presence of carbonic anhydrase, so that the action of CO_2 is much greater in the cells containing this enzyme. Hence the change in excitability observed in the behavior of the L ganglion cell when the % CO_2 is increased cannot be explained by direct action of the CO_2 on the bipolar cell but as a secondary effect on the latter, resulting from the hyperpolarization of the membrane of the horizontal cell (Fig. 3, CO and LM). If we assume a nonspecific effect of the CO_2 in the membrane of the bipolar cell, this would result in a change of potential in the entire membrane, and this would not produce an electrical dipole oriented as required to explain the changes in transretinal d.c. potential produced by varying the % CO_2. Such changes depend on variations in the polarization of the membrane of the dentritic terminals of the bipolars, which are secondary to the transferaptic influence of the horizontal cells.

The regulatory action of CO_2 on the excitability of the nervous system in general does not depend on the effect of CO_2 on the neuron, where we find no carbonic anhydrase (Giacobini, 1962; Parthe, 1967), but on the primary and direct effect of CO_2 on other structures which contain this enzyme and which are capable of influencing the neurons (Laufer et al., 1961; Negishi and Svaetichin, 1966 a).

By means of this transferaptic mechanism the horizontal cells control the excitability of the dendrites of the bipolar cells, and the changes in excitability occurring in the type L ganglionic cells depend exclusively on such nonspecific control. The type L ganglion cell (Fig. 1 and 2) apparently transmits information about the brightness of the stimulus, which is an additive function. In accordance with this, inhibitory effects have not been observed in these cells, which is due /271 to the absence of specific control (see below) on the part of the amacrine cell system, according to an earlier suggestion (Svaetichin et al., 1961).

The membrane potential level of the horizontal cells holds a value of about 10 mV in retinas adapted to darkness, while in light-adapted material the potential is close to 35 mV (Mitarai et al., 1961). The results of these experiments have been confirmed and are illustrated with the recordings in Fig. 4, F-K, and in the lower part of diagram Q. The change occurring in the adaptation of the retina in passing from darkness to light is related to an increase in potential recorded across the receptor layer (Fig. 4, A-E) and also to an increase in the resting level of the medial and internal horizontal cells (Fig. 4, F-K). In this case the function of the rods is replaced by that of the cones and there occurs simultaneously a change in spectral sensitivity similar to the Purkinje phenomenon. This fact also indicates a correlation between the retinal excitability and the potential level of the networks formed by the horizontal cells.

In a recent publication Witkowsky (1967) reports that he could not confirm the sequence of adaptational changes occurring in the medial and internal horizontal cells observed by Mitarai et al. (1961) and illustrated in Fig. 4, F-K and Q. The reason for not being able to confirm the results of Mitarai et al. (1961) surely does not depend on the use of "tropical fishes", since Mitarai (1961) has confirmed them with "Japanese fish" (carp). The reason is probably based on the fact that Witkowsky (1967) only studied the external horizontal cells, which do not show such adaptational changes, according to Mitarai et al. (1961).

SPECIFIC CONTROL

Opposed Signals of Color and Black-White

Electrophysiological studies on the patterns of impulses of the ganglionic cells (Granit and Svaetichin, 1939; Wagner et al., 1960) and in the lateral geniculate body of the monkey (de Valois et al., 1967), on the S-potential (Svaetichin, 1953, 1956; Svaetichin and MacNichol, 1958; Svaetichin et al., 1961, 1965 a, b), and microspectrophotometry of the photopigments of individual cones (Marks et al., 1964; Marks, 1965; Svaetichin et al., 1965 b) show that the two classical theories of color vision can be fused into a single theory, that of Young-Hering (Svaetichin et al., 1965 b). The chromatic modality (type C) of S-potentials is characterized by a depolarization or hyperpolarization of the membrane, depending on the wavelength of the light stimulus (Fig. 1, right: AmS, AmIe, AmIi, AmP1, and Fig. 2, Recordings IV, VIII, IX, and XIII). The "curves of spectral responses" recorded in the different varieties of amacrines show total agreement with the diagrammatic representations of the opponent color systems suggested by Hering. These observations indicate that a pair of amacrines (Fig. 2, AmS-AmIe and AmIi-AmP1) generates the different chromatic responses on the basis of the information coming from the three classes of cones, and in this way influence the coding of opponent colors in the ganglionic cells specialized for it.

Witkowsky (1967, p. 558) indicates that in the retina of the carp: "Ganglion   /272 cells may be either "on" to short wavelength stimulation and "off" to long, or reverse, but C-potentials [are] invariably hyperpolarized to short wavelengths and depolarized to long ones." S-potentials of type C of both polarities like mirror images have been described (Svaetichin, 1956; Svaetichin et al., 1961, 1965 a, b) and are illustrated in Fig. 1, on the right (AmS and AmIe; AmIi and AmP1). The potentials of the type marked AmIe in Fig. 1 are relatively easily obtained in the retina of Mugil brasiliensis due to the large size of the interstitial amacrine cells in this species, while this modality of chromatic response is difficult to record in the retina of other species; undoubtedly they can be obtained by using superfine electrodes. The inability of Witkowsky (1967) to confirm the existence of C-type S-potentials of reversed polarity as well as the adaptational changes in the medial and internal horizontal cells (see above) does not favor his final conclusion (p. 560): "that ganglion cells respond independently of S-potentials for the stimulus parameters tested."

Since for the psychology black-white a quality equivalent to color, it is also suggested that the code for black-white depends on a pair of amacrines which generate opposed signals. Intracellular recordings in certain ganglion

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cells show square waves which can be depolarizing or hyperpolarizing, depending on the wavelength of the light stimulus (Fig. 2, Recording X). This observation indicates that the square waves recorded are related to the square and opponent responses of the C-type in the membranes of the amacrines. It has also been noted that the opponent behavior of the membrane potential of particular ganglion cells (Fig. 2, B-W) depends on the white light projected on focal points (in the area of the electrode) and nonfocal points (Fig. 2, Recording XI). Recordings similar to Recording IX were obtained by Wiesel (1959) in the retina of the cat. It is suspected that this class of ganglionic cells codifies the information for black-white. As was demonstrated earlier, the horizontal cells exercise control over the dendrites of the bipolar cells; analogously it is concluded that a pair of amacrines acts on the dendrites of certain ganglion cells localized in a particular stratum of the internal plexiform layer (Fig. 2). We do not intend to set forth here in all fullness our theory of specific amacrine control over ganglion cells, but the fundamental idea of this theory is presented in the diagram of Fig. 2 (see Svaetichin et al., 1965 b). The intracellular recordings obtained in the ganglion cells (Fig. 3, Recordings X and XI) are interpreted as a consequence of two parallel influences on these cells: 1) the synaptic effect which produces excitatory post-synaptic potentials of the "on" and "off" type and on which the impulses are superimposed, and 2) the specific control of the S cells, which is provided transferaptically by a pair of functionally coupled amacrines (for example, AmS and AmIe and Recordings VIII and IX), square waves of opposite polarity then appearing, which reflects the graduated control of the excitation (depolarization, discharge "on") and inhibition (hyperpolarization, discharge "off").

The example given below helps understand the manner in which the specific control system is conceived to operate. A light stimulus of 638 nm elicits a hyperpolarization of AmIe (Fig. 2, Recording IX, second response), which in turn induces transferaptically a depolarization of the ganglion cell Y (Fig. 2, Recording X, second response), thus determining the magnitude of the "on" discharge, caused by the synaptic bombardment of the bipolar cell. The same light stimulus of 638 nm causes a concomitant depolarization of AmS (Fig. 2, Recording VIII, second response), which transferaptically induces a hyperpolarization of ganglion cell B, resulting in inhibition in "on" and discharge of impulses in "off" (similar to the response of ganglion cell Y to a stimulus of 459 nm, Fig. 2, Recording X, first response). In this opponent system for the reciprocal control of excitation-inhibition, the two (B and Y) parallel channels (receptor-bipolar-ganglion cell)should be coupled, and the coupling would occur at the level of the amacrine cells.

The process of equalization of colors requires a mechanism capable of converting information derived from different classes of cones into positive and negative signals, which are added algebraically; the operation of coupled pairs of amacrine cells (AmS-AmIe and AmIi-AmP$_1$) apparently represents the mechanism for color equalization. In view of the fact that the networks of amacrine cells operate as functional units, the spatial contrast of color and the phenomenon of induction (Land effect) can also be explained in terms of coupled systems of amacrine cells.

In conclusion, the control system which operates in the retinal gray matter as discussed in this paper confirms the theory delineated in earlier papers from

this laboratory, and this permits us to suggest that the functioning of the cere- /274
brum is based on similar mechanisms of interaction between neurons conducting
impulses and control cells.

Acknowledgment

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Addendum

Comparative morphological and electrophysiological studies show that the **C-type S-potentials indicated** by AmIe and AmIi in Fig. 1 are observed in interstitial amacrine cells. These types of responses are easily obtained in the retina of Mugil brasiliensis, an unusual and unique species among the teleosts studied, which possesses giant-size cells of this type. On the other hand, the origin of the responses designated AmS and AmP1 in Fig. 1 is still tentative. The responses of the AmS type are recorded immediately below the horizontal cells, and it has been considered that they originate in the stellate amacrine cells (Fig. 1). Recent observations on the structure of the retina (Parthe, 1967 b; O'Daly, 1967 b) make it more probable **that the S-potentials of the AmS** type originate in pyriform amacrine cells which ramify in stratum 1 of the internal plexiform layer. If this is the case, **all the different S-potentials of the C-type** would originate in amacrine cells with ramifications distributed exclusively in the strata of the internal plexiform layer.

Recent studies by Parthe (1967 b) show that the displaced ganglion cells, called Dogiel cells by Cajal, are abundant in the external zone of the internal plexiform layer of the retina of the teleosts. Axons of these cells have been observed to cross the internal plexiform obliquely in their path to the layer of optic nerve fibers. Part, if not all, of the myelinated axons positioned tangentially in the external zone of the plexiform originate in the Dogiel cells mentioned. In view of these findings and keeping in mind that the dendrites of ganglion cells are fine in the external zone of the internal plexiform, it is suspected that the transitory potentials at the "on" and "off" of the stimulus (Fig. 3 "d"), which have been interpreted in this paper as generated in dendrites of type-L ganglion cells, actually are recordings of the Dogiel cells, conclusions which agree with the observations of Byzov (1965) and Tomita (1963).